



Global Research & Development

February 14, 2005

Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane, rm. 1061
Rockville, Maryland 20852

Re: Draft Guidance for Industry on the Role of Human Immunodeficiency Virus Drug
Resistance Testing in Antiretroviral Drug Development
[Docket No. 2004D-0484, 69 *Federal Register*, 69374-69375, November 29, 2004]

Dear Dockets Management:

Pfizer submits these comments on the Draft Guidance for Industry on the Role of Human Immunodeficiency Virus Drug Resistance Testing in Antiretroviral Drug Development, published in the *Federal Register* on November 29, 2004.

Pfizer appreciates this opportunity to provide comments and commends the Agency for developing guidance on this topic. As well, if considered valuable, we would invite direct dialogue with the Agency.

Our comments are attached.

Sincerely,

A handwritten signature in black ink, appearing to read "JH HA".

Jennifer Hammond, Ph.D.
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Pfizer Global Research and Development

2004D-0484

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Comments to Docket No. 2004D-0484
Draft Guidance for Industry, *Role of HIV Drug Resistance Testing in*
***antiretroviral Drug Development*, November 2004**

Page 4, Line 160

“In vitro selection of resistant HIV-1 variants, the phenotype and genotypic characterization of resistant viruses, and cross-resistance analyses should be examined prior to initiation of clinical studies in HIV-infected patients.”

Comments:

- The need to assess in vitro resistance prior to the initiation of clinical studies in HIV infected patients is driven by the need to understand the risk that resistant variants will emerge in patients participating in short-term studies. For compounds with a low genetic barrier to resistance, resistant variants will emerge rapidly in vitro, enabling the characterization of resistant virus and cross-resistance to other agents in the same class prior to the initiation in clinical studies in HIV-infected patients. Compounds with high-genetic barriers to resistance, however, may require many months to select for resistant variants, and these experiments may not be fully completed prior to the initiation of studies in HIV infected patients.
- FDA should clarify the in vitro resistance data required in order to proceed with studies in HIV-infected patients.

We propose the above text be changed to the following:

“In vitro selection of resistant HIV-1 variants, the phenotype and genotypic characterization of resistant viruses, and cross-resistance analyses should **at a minimum be examined ongoing prior to at the time initiation of clinical studies in HIV-infected patients are initiated. Data available at the time clinical studies in HIV-infected patients are initiated should enable an accurate assessment of the risk that resistant variants will emerge in patients participating in short-term studies, and to determine if the investigational drug can be safely administered as a single agent for a limited period of time.**”

Page 4, Line 183

“A well-characterized wild-type HIV laboratory strain grown in peripheral blood mononuclear cells should serve as a reference standard.”

Comment:

- Depending on the mechanism of action of the investigational compound, growing a well-characterized HIV laboratory strain in PBMCs may not be necessary.

We propose the above text be changed to the following:

~~“A well-characterized wild-type HIV laboratory strain grown in peripheral blood mononuclear cells should serve as a reference standard.”~~

Pages 4-5, Line 188

“Because of genetic variation, determination of antiviral activity against a broad spectrum of viruses (50-100 well-characterized laboratory strains and clinical isolates of HIV) is recommended.”

Comment:

- Evaluating the antiviral activity of an investigational agent against 50-100 strains is excessive, particularly for compounds inhibiting well-conserved targets (e.g. protease, reverse transcriptase or integrase). Antiviral activity against 25-50 strains should be sufficient to establish the effect of genetic variation on a drug. If results from this initial study indicate genetic diversity has a significant impact on drug activity, follow-up studies with additional strains of HIV may be warranted.

We propose the above text be changed to the following:

“Because of genetic variation, determination of antiviral activity against a broad spectrum of viruses (**a minimum of 25-50** ~~50-100~~ well-characterized laboratory strains and clinical isolates of HIV) is recommended.”

Page 5, Line 197

“The effects of 45-50% human serum and/or human plasma plus a-acid glycoprotein on the in vitro antiviral activity of the investigational drug should be evaluated for multiple laboratory and clinical isolates, and serum-adjusted IC₅₀ values should be determined.”

Comments:

- The relationship between protein binding and in vivo antiviral activity for antiretroviral agents has not been fully established, particularly for the NRTI, NNRTI and entry inhibitor classes of compounds.
- Assays evaluating the antiviral activity of a compound in the presence of 50% human serum can yield highly variable results, complicating interpretation of the data and an accurate assessment of the effects of protein binding on the antiviral activity of a compound. A more accurate method of measuring the effect of protein binding on the antiviral activity of a compound is to measure the amount of “free” (unbound) drug in tissue culture media supplemented with 10% fetal bovine (or calf) serum by HPLC or equilibrium dialysis. The serum-free IC₅₀ can subsequently be determined (Hickman, 2004).

- Cell-based assays utilizing 45-50% human plasma may not be technically feasible (cytotoxicity, presence of clotting factors in plasma).
- Data published by Molla et al. (Molla, 1998), as well as our own experience with protein binding assays, indicate that the effect of protein binding on the antiviral activity of a compound is consistent between virus strains and cell types. There is thus little utility in conducting protein binding assays with an investigational drug with multiple laboratory and clinical isolates.

We propose the above text be changed to the following:

“The free fraction of the investigational drug in tissue culture medium supplemented with 10% fetal bovine (or calf) serum should be determined using HPLC or equilibrium dialysis and the serum-free IC₅₀ value should be determined. Alternatively, the effects of 45-50% human serum ~~and/or human plasma plus alpha-acid-glycoprotein~~ on the in vitro antiviral activity of the investigational drug should be evaluated for ~~multiple laboratory and clinical isolates~~ **at least one well-characterized laboratory isolate, and the serum-adjusted IC₅₀ values should be determined.**”

Page 5, Line 207

“Selection of variants resistant to the investigational drug should be repeated several times to determine if the same or different patterns of resistance mutations develop.”

Comment:

- It is not clear in the document if the selection of resistant variants should be repeated with the same strain or if the selection can be repeated using a different strain of virus. Repeating the selection with a different strain of virus can provide the opportunity to evaluate the effect of genetic background on the pattern of resistance.

We propose the above text be changed to the following:

“Selection of variants resistant to the investigational drug should be repeated ~~several times~~ **twice using the same strain or conducted once with two different strains** to determine if the same or different patterns of resistance mutations develop.”

Page 5, Line 211

“Two basic methods have been developed to identify mutations conferring a reduction in susceptibility to a drug.”

Comment:

- The first method described is useful for identifying compounds with low genetic barriers to resistance. Consistent with our comments on **line 160**, we

would recommend that this method be completed prior to the initiation of studies in HIV-infected patients, while the second method should be ongoing at the time studies in HIV-infected patients are initiated. The second method should only be used when the first method fails to select for drug-resistance mutations.

Page 5, Line 219

“In the second method, virus is passaged in the presence of increasing drug concentrations starting at twice the IC₅₀ value for the parent virus.”

Comment:

- The appropriate concentration to initiate in vitro serial passage experiments is dependent on several characteristics of the drug, including potency and genetic barrier to resistance. A 2X IC₅₀ starting concentration may be too high for some drugs with a very high genetic barrier to resistance or very high potency. In order to generate strains of HIV resistant to a given drug, starting concentrations may need to be reduced.

We propose the above text be changed to the following:

“In the second method, virus is passaged in the presence of increasing drug concentrations starting ~~at twice~~ **near** the IC₅₀ value for the parent virus.”

Page 5, Line 229

“Once mutations are identified, their ability to confer phenotypic resistance should be evaluated in a recombinant virus system (e.g., by using SDM or PCR amplification of relevant portions of the virus genome to introduce these mutations into a standard laboratory genetic background).”

Comments:

- While recombinant virus systems have been used successfully to characterize mutations conferring resistance to reverse transcriptase and protease, other systems may be more appropriate for characterizing resistance to inhibitors with other mechanisms of action (e.g. entry inhibitors).
- During in vitro serial passage, many mutations may appear, but not all may be necessary for the drug-resistant phenotype. To avoid the unnecessary generation and characterization of multiple SDM-derived mutants, we suggest that passaged virus be characterized first, followed by SDM to identify the mutations sufficient to reproduce the level of resistance observed in the passaged virus.

We propose the above text be changed to the following:

“Once mutations are identified, their ability to confer phenotypic resistance should be evaluated in a recombinant virus system (e.g. by using site-directed mutagenesis or polymerase chain reaction (PCR) amplification of relevant portions of the virus genome to introduce these mutations into a standard laboratory HIV genetic background) **or other suitable system, such that the mutations necessary to reproduce the resistant phenotype are identified.**”

Page 6, Line 254

“Recombinant viruses containing drug resistance associated mutations to an investigational drug should be tested for susceptibility to approved and investigational drugs of the same class. Conversely, laboratory strains and 10-30 well-characterized clinical isolates containing resistance-associated mutations for each of the approved and investigational members of the same class should be tested for susceptibility to the investigational drug.”

Comments:

- Obtaining material for other investigational compounds can be very difficult (due to cost, licensing issues, etc.).
- Information concerning the mutations that confer resistance to other investigational agents of the same class may not be publicly available.
- Information may be published on compounds no longer in clinical development or at early stages of development (pre-Phase 2b/3). Obtaining these compounds and generating SDM viruses would incur a significant cost in time and resource with little benefit.

We propose the above text be changed to the following:

“Recombinant viruses containing drug resistance associated mutations to an investigational drug should be tested for susceptibility to approved and, **where possible**, investigational drugs of the same class **In Phase 2b/3 development**. Conversely, laboratory strains and 10-30 well-characterized clinical isolates containing resistance-associated mutations for each of the approved and, **where possible**, investigational members of the same class **in Phase 2b/3 development** should be tested for susceptibility to the investigational drug.”

Page 9, Line 392

“In addition, continuation of resistance monitoring on subsequent regimens is important, where applicable.”

Comment:

- Continuing to follow and collect samples for resistance testing from patients no longer enrolled in a study can be very challenging. In many cases, patients may elect to enter into a new experimental protocol, and the sponsors of the new protocol may prohibit the collection of samples for resistance testing.

We recommend eliminating this section from the guidance document, or making this a requirement only for agents being evaluated in treatment naïve patients.

Page 13, Line 499

“Agreement on susceptibility breakpoints for most antiretroviral agents is limited; therefore, the median-fold change in susceptibility can be used as a breakpoint.”

Comment:

- Typically, the assay-specific cutoff is used as a breakpoint for resistance (4.0-fold for Virco, 2.5-fold for ViroLogic).

We propose the above text be changed to the following:

“Agreement on susceptibility breakpoints for most antiretroviral agents is limited; therefore, the median fold-change in susceptibility **or assay-specific cutoff** can be used as breakpoints.”

Page 14, Line 540-546

“Phase 3 trials should incorporate prospective rollover designs to provide for assessment of virologic responses in study subjects administered subsequent antiretroviral regimens.”

Comment:

- The request to assess "salvageability" after failure in Phase III trials would be difficult to reliably address and, in some cases, impossible to fulfill. Any such analyses would be deeply confounded by the heterogeneity of the salvage regimens. In addition, for agents that target deep salvage, there may simply be no other viable options at failure.

We recommend eliminating this section from the guidance document, or making this a requirement only for agents being evaluated in treatment naïve patients.

Pages 20-22, Various Locations

Comment:

- Genotype, phenotype and tropism data cannot be accurately determined for endpoint isolates when the patient’s viral load is <500 copies/mL.

We recommend that the criteria for testing endpoint isolates be clearly delineated in the text.

Appendix B: Genetic Threshold for Resistance

Comments:

- We feel it would be helpful if the differences between compounds with low and high genetic barriers to resistance were more clearly defined in the document.
- Typically, compounds with low genetic barriers to resistance rapidly select for single or double mutations that confer high-level resistance to the drug without negatively impacting the replicative fitness of the virus.
- Compounds with high genetic barriers to resistance select for resistance mutations after numerous serial passages in the presence of drug. In many cases, multiple mutations are required for high-level resistance to the drug. It is possible, however, for individual mutations that negatively impact the replicative fitness of the virus but confer high-level resistance to the drug to be selected only after extensive passage of virus in culture. In these cases, additional compensatory mutations are required in order to restore the replicative fitness of the virus.

We propose the following changes to the text in Appendix B:

Lines 872 and 890

"1. Single or double mutations appear after selection for a limited number of passages and a limited number of days in cell culture."

"1. **Single or** multiple mutations appear after serial passages of HIV-1 in cell culture in the presence of increasing concentrations of the agent **over an extended period of time.**"

Lines 874 and 892

"2. Insertion of the mutation(s) by site-directed mutagenesis into standard laboratory strains yields ~~strains~~ **virus** with reduced susceptibility (**>10-fold**) and **near wild-type replicative fitness.**

"2. Insertion of **individual** mutations by site-directed mutagenesis into standard laboratory strains **does not** yields ~~strains~~ **virus** with **>10-fold** reduced susceptibility **combined with near wild-type replicative fitness. Multiple mutations must be introduced to yield strains with >10-fold reduced susceptibility and near wild-type replicative fitness.**"

References

Molla, A., S. Vasavanonda, G. Kumar, H.L. Sham, M. Johnson, B. Grabowski, J.F. Denissen, W. Kohlbrenner, J.J. Plattner, J.M. Leonard, D.W. Norbeck, and D.J. Kempf. 1998. Human serum attenuates the activity of protease inhibitors toward wild-type and mutant human immunodeficiency virus. *Virology* 250: 255-262.

Hickman, D., S. Vasavanonda, G. Nequist, L. Colletti, W. Kati, R. Bertz, A. Hsu, and D.J. Kempf. 2004. Estimation of serum-free 50-percent inhibitory concentrations for human immunodeficiency virus protease inhibitors lopinavir and ritonavir. *Antimicrob Agent Chemother* 48: 2911-2917.